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Microgaster godzilla (Hymenoptera, Braconidae, Microgastrinae), an unusual new species from Japan which dives underwater to parasitize its caterpillar host (Lepidoptera, Crambidae, Acentropinae)

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Abstract

A new species of Microgastrinae (Hymenoptera: Braconidae) parasitoid wasp, *Microgaster godzilla* Fernandez-Triana & Kamino, is described from Japan. From a biological and morphological perspective this is a very unusual species. It represents only the third known microgastrine to be aquatic, and the first one to be found entering the water. The female wasp searches for its hosts, aquatic larvae of *Elophila turbata* (Lepidoptera: Crambidae), mostly by walking over floating plants, but occasionally diving underwater for several seconds to force the larva out of its case, when it is quickly parasitized (parasitization was always observed above water). The unique searching behaviour of *M. godzilla* as well as its parasitization of aquatic larvae was filmed and it is presented here. The wasp has simple tarsal claws, which are elongate and strongly curved, similar to those found in the related genus *Hygroplitis*; they seem to represent an adaptation for gripping to the substrate when entering the water. The new species is described based on morphological, molecular (DNA barcoding), biological and ethological data. Additionally, we provide detailed diagnoses to recognize *M. godzilla* from all other described species of *Microgaster* and *Hygroplitis* in the Palearctic, Nearctic and Oriental regions.

Keywords
aquatic parasitoid, biology, Lepidoptera, Microgastrinae, Nearctic, Oriental, Palearctic, parasitoid behaviour, taxonomy

Introduction

The order Hymenoptera comprises relatively very few aquatic species, with only 150 species within 11 families (0.13% of all described Hymenoptera species) recognized so far (Bennett 2008). For a definition of aquatic Hymenoptera we follow that applied by Bennett (2008: 529): "*…species in which female adults enter the water to search for hosts, those with endoparasitoid larvae inside aquatic larval hosts (even if oviposition is terrestrial); and those in which freshly eclosed adults must travel to the water's surface following pupation (even if they develop inside stems of emergent vegetation)*".

The Braconidae subfamily Microgastrinae is one of the most speciose groups of parasitoid wasps, and probably the single most important group of Lepidoptera parasitoids (Fernandez-Triana et al. 2020). However, only two species had been recorded so far as being aquatic, *Dolichogenidea amaris* (Nixon, 1967) and *Hygroplitis rugulosa* (Nees, 1834) (Bennett, personal communication); in both cases the wasp are parasitizing lepidopteran larvae which are aquatic (Morley 1936; You et al. 1988; Litsinger et al. 1994). This paper describes a new *Microgaster* from Japan, which constitutes the third known species of aquatic Microgastrinae.

Methods

Wasp specimens were reared from larvae of *Elophila turbata* (Butler, 1881) (Lepidoptera: Crambidae, Acentropinae) collected in ponds covered with water ferns, *Azolla* spp. (Salviniaceae), in several cities of the Osaka and Kyoto Prefectures, Honshu, Japan, between 2015 and 2017. The lepidopteran species was identified by one of the authors of this paper (Y. Yoshiyasu).

Emerged adult wasps were kept in laboratory conditions for observation of the wasp's parasitization biology. Each individual female was placed in a plastic cup of approximately 30 mL volume, with moistened filter paper at the bottom of the container. The wasps were fed drops of 100% honey and kept at two different temperatures (12 °C and 25 °C).

We observed the oviposition behavior of the female wasps in a small plastic aquarium (10 × 20 × 10 cm). Twenty host larvae and one female wasp were released in the aquarium together. Observations of the wasp were taken with a Canon iVIS HF S21 video camera. The observations were repeated five times using different host and wasp individuals.

The holotype of the new species is deposited in the Osaka Prefecture University (**OPU**) collection in Sakai, whereas paratypes are deposited at OPU and the Canadian National Collection of Insects (**CNC**) in Ottawa.

For the species description the morphological terms and measurements of structures are mostly as used by Mason (1981), Huber and Sharkey (1993), Whitfield (1997), Karlsson and Ronquist (2012), and Fernández-Triana et al. (2014). We studied the new species alongside all described species of *Microgaster* and the related genus *Hygroplitis* (list of species from Yu et al. 2016 and Fernandez-Triana et al. 2020) from the Palearctic, Nearctic and Oriental regions. The new species was compared against authenticated specimens in the CNC collection, as well as original descriptions and/or taxonomic revisions of those two genera (Muesebeck 1922; Wilkinson 1929; Nixon 1968; Papp 1976; Tobias 1986; Austin and Dangerfield 1992; Shaw 1992, 2004, 2012; Chen and Song 2004; Kotenko 2007; Xu et al. 2007).

Photos of the preserved specimens were taken with a Keyence VHX-1000 Digital Microscope, using a lens with a range of 13–130×. Multiple images through the focal plane were taken of a structure and these were combined to produce a single in-focus image, using the software associated with the Keyence System.

We DNA barcoded some specimens, using the standard animal locus of the 5' region of the cytochrome c oxidase I (COI) gene (Hebert et al. 2003). Briefly, DNA extracts were obtained from single legs using a glass fibre protocol (Ivanova et al. 2006), and total genomic DNA was re-suspended in 30 µl of dH2O. The barcode region, 658 base pairs (bp) region near the 5' terminus of the COI gene, was amplified using standard primers following established protocols (see Smith et al. 2008). All information for the sequences associated with each individual specimen barcoded (including primers and trace files) is available on the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007). The Barcode Index Number (BIN) is used here to discuss species limits, following the BIN concept detailed in Ratnasingham and Hebert (2013). DNA barcodes from the specimens used in this paper were compared with 960+ DNA barcodes of *Microgaster* and *Hygroplitis* with at least 400 bp available in BOLD as of September 2020.

Results

***Microgaster godzilla*, Fernandez-Triana & Kamino, sp. nov.**
http://zoobank.org/4ED57AC8-A50E-4995-AA72-C55FF96DD31A
Figs 1, 2

Material examined. ***Holotype.*** JAPAN • ♀, OPU; Honshu, Osaka Prefecture, Sakai City, Shoubu-ike Pond, 1.v.2017, ex. *Elophila turbata* on 13.v.2017, T. Kamino, voucher code: CNC924596. ***Paratypes.*** JAPAN • 1 ♀, 1 ♂, CNC; Honshu, Osaka Prefecture, Moriguchi City, Yagumo-Higashimachi, 26.i.2017, T. Kamino, voucher codes: CNC924597, CNC924599, 1 ♂ OPU; same locality and date than holotype, voucher code: CNC924598; 1♀, OPU; Osaka Prefecture, Moriguchi City, Niwakubo, 30.ix.2016; 1♀, 1♂, OPU; Kyoto Prefecture, Kyoto City, Shimogamo, 15.ix.2017.

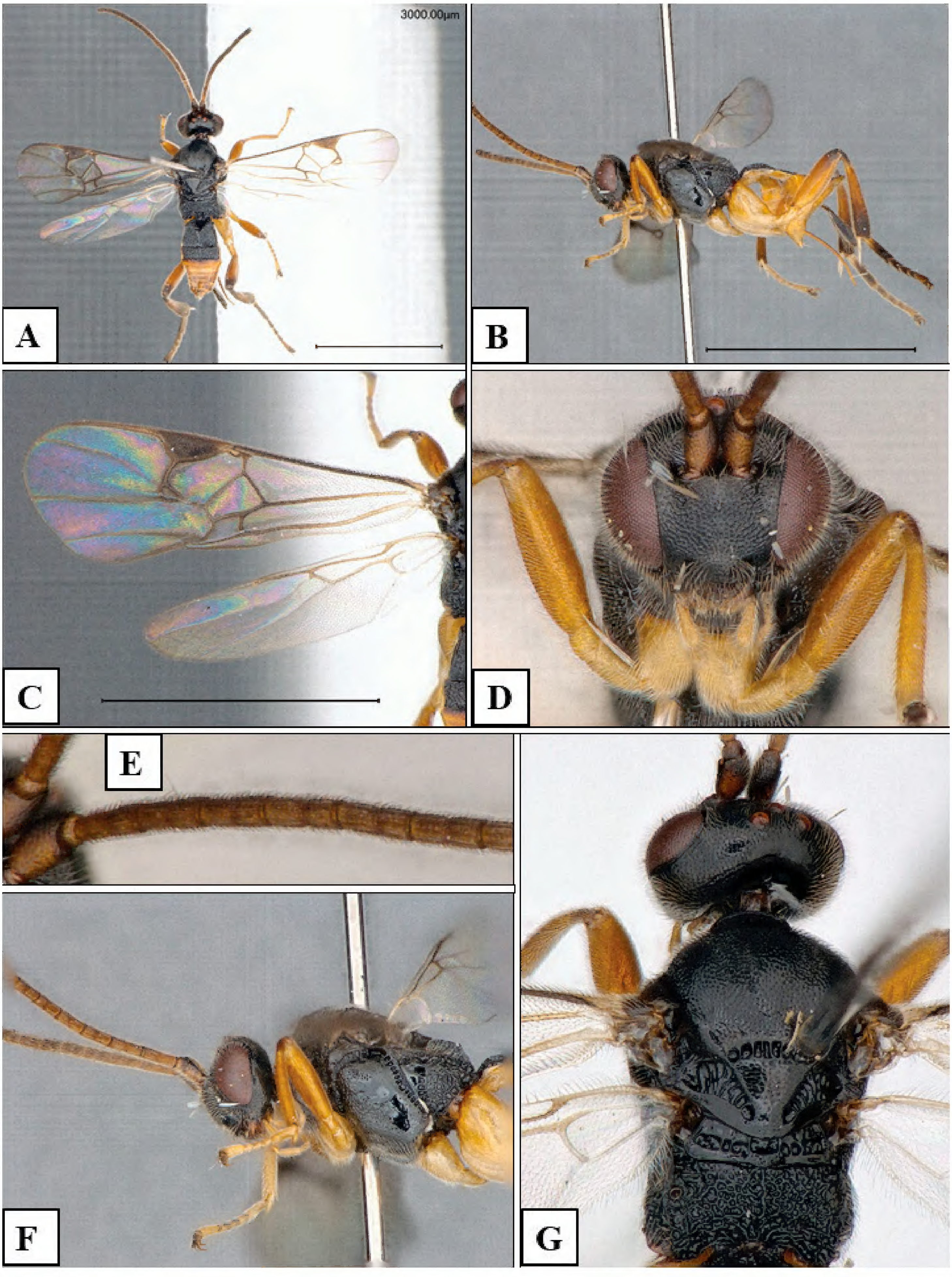

Figure 1. *Microgaster godzilla*, female holotype. **A** habitus dorsal **B** habitus lateral **C** wings **D** head, frontal **E** details of antenna **F** head and mesosoma, lateral **G** head and mesosoma, dorsal.

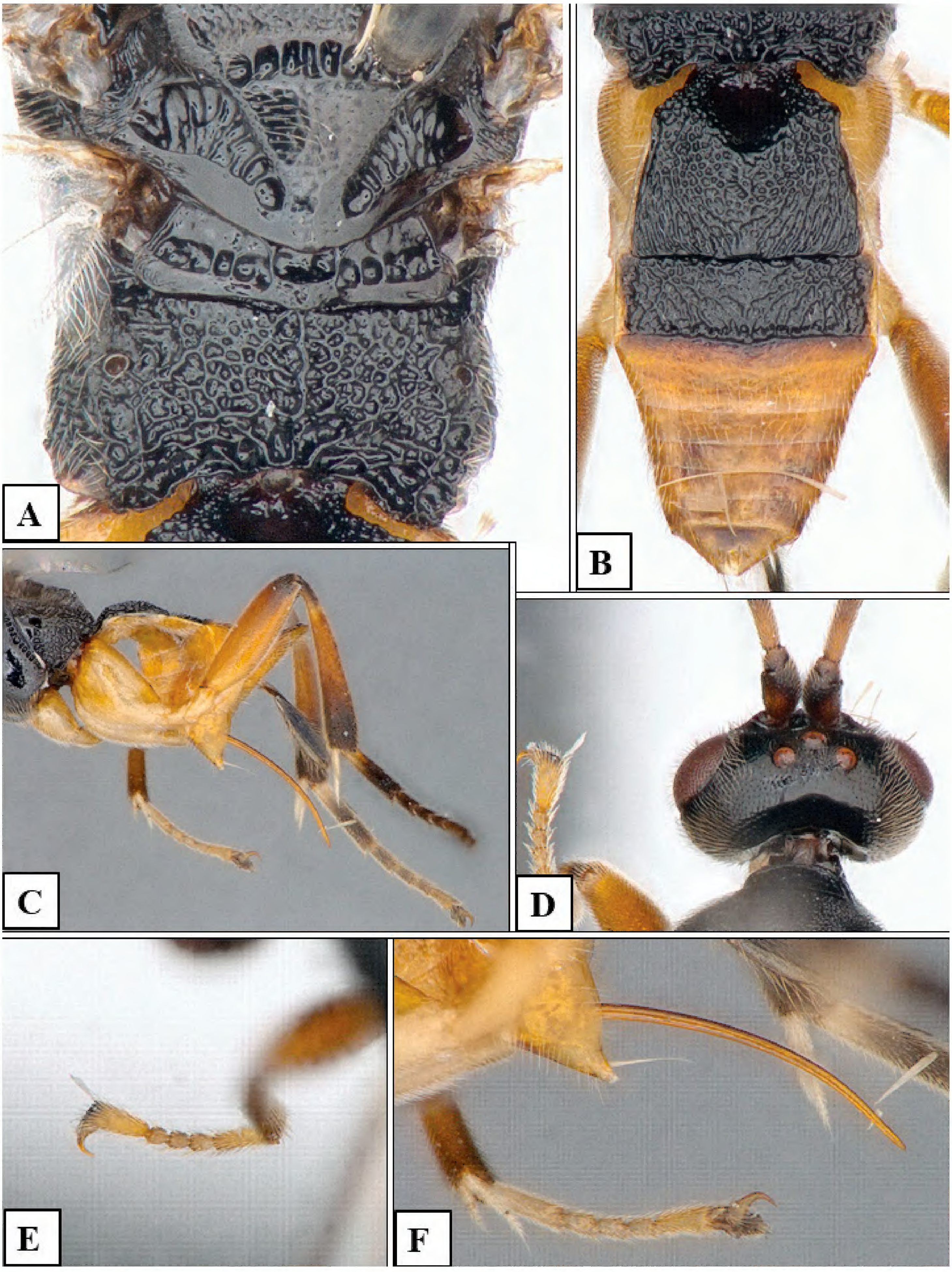

Figure 2. *Microgaster godzilla*, female holotype. **A** details of scutellar complex and propodeum **B** metasoma, dorsal **C** metapleuron and metasoma, lateral **D** details of ocelli dorsal **E** tarsal claw **F** hypopygium and ovipositor.

Diagnosis. Among all described species of *Microgaster* this species can be distinguished because of its unique combination of morphological characters. Color patterns are distinctive, especially having all legs almost entirely yellow (only apical 0.1 of metafemur, apical 0.3 of metatibia and metatarsus are dark brown to black), tegula dark brown, pterostigma brown, and metasoma dorsally with T1–T2 black and T3+ orange-yellow. Very few *Microgaster* species have all coxae yellow, and then the color of the tegula and/or metasoma dorsally is usually different. Beyond color, the combination of flagellomeres with relatively distinct setae (bristly), eyes convergent ventrally, face dull due to transverse, rugose sculpture (including indication of vermiculate rugosities towards sides), notauli barely marked by impression or sculpture (and overall sculpture of anteromesoscutum with fine and relatively shallow punctures), scutellar disc mostly smooth, mesopleuron without strong crenulated sulcus, T2 and T3 about same length, T3+ smooth, all tarsi with last segment enlarged, and large but simple tarsal claws are also of diagnostic value. *Microgaster godzilla* shares some features with the described species of *Hygroplitis*, most of which also have light-coloured legs, including all coxae in many species, large tarsal claws, and last segments of tarsi enlarged. However, most *Hygroplitis* species have the body depressed, notauli are strongly marked, antennae have three rows of placodes and the mesopleuron has a strong, crenulated sulcus.

Although there is no available key that covers all described *Hygroplitis* and *Microgaster*, we found that all Palearctic, Nearctic and Oriental species previously described in those two genera differ from the diagnosis provided above for *M. godzilla* by at least one (usually more) characters. To facilitate future work on the genus we provide one-to-one comparisons of *M. godzilla* with every other previously described species of those two genera in the biogeographical regions relevant to the new species (see Suppl. material 5 for detailed comparisons).

Description. ***Color***. Head and mesosoma black; metasoma dorsally with T1–T2 black, T3+ orange-yellow; metasoma ventrally entirely orange-yellow, including hypopygium; antenna light brown; palpi orange-yellow; tegula and humeral complex dark brown; all legs almost entirely yellow (only apical 0.1 of metafemur, apical 0.3 of metatibia and metatarsus are dark brown to black); metatibial spurs white; pterostigma and most veins brown. ***Head***. Eyes convergent ventrally; face dull due to transverse, rugose sculpture (including indication of vermiculate rugosities towards sides); frons with transverse striation; gena mostly smooth; space between ocelli with weak sculpture (a short but vague carina is barely defined); flagellomeres bristly, with relatively distinctive setae. ***Mesosoma***. Overall sculpture of anteromesoscutum with fine and relatively shallow punctures; notauli barely marked by impression or sculpture; scutoscutellar sulcus deep and broad, with strong crenulae; scutellar disc mostly smooth, with very shallow and sparse punctures; propodeum entirely sculptured with coarse reticulation and a median, longitudinal carina; mesopleuron mostly smooth and shiny on dorsal third, mostly sculptured on ventral 0.6, without strong, crenulated sulcus; metapleuron coarsely sculptured. ***Metasoma***. T1 and T2 strongly sculptured; T3+ smooth; T1 very broad, its width at posterior margin twice that at anterior margin,

its median length 0.9 its width at posterior margin; T2 rectangular and about same length as T3; hypopygium mostly inflexible, with a weakly defined and small fold apico-ventrally; ovipositor sheaths with relatively long setae the setose part roughly half the length of metatibia. ***Legs***. All legs with simple and large tarsal claws; all tarsi with last segment enlarged. ***Wings***. Fore wing with relatively large and quadrangular areolet, vein R1 longer than pterostigma length, vein r longer than vein 2RS and much longer than areolet height, vein r arising beyond mid point of pterostigma; hind wing with vannal lobe entirely setose. ***Body measurements*** (in mm). Body length: 3.3; fore wing length: 3.2; ovipositor sheath length 1.28; metatibia length 0.62; metafemur length/width: 0.99/0.28; flagellomere 2 length/width: 0.26/0.07; flagellomeres 13+ missing in the two female specimens (holotype and one paratype) available to JFT for study and species description.

Distribution. The species has only been collected in the prefectures of Osaka and Kyoto in Honshu, Japan.

Biology. *Microgaster godzilla* sp. nov. has been reared from *Elophila turbata* (Butler, 1881) (Lepidoptera: Crambidae, Acentropinae), whose larvae are aquatic. Each larva constructs a portable case from fragments of aquatic plants (e.g. *Azolla* spp., *Trapa* spp. and *Spirodela* spp.) and lives inside the case which is found slightly above or slightly under the water surface (Yoshiyasu 1985). Females of *M. godzilla* walk over the floating plants while searching for hosts. Once the wasp finds a case, it repeatedly probes it with its antennae and moves around, eventually forcing the larvae to come out of the case, when it is parasitized by the wasp by quickly inserting its ovipositor. In some cases, *M. godzilla* completely dives underwater for several seconds, in order to search for the submerged caterpillar and force it out of the case. In all cases we observed, oviposition occurred above water, where the host larvae went trying to escape the wasp (Suppl. material 1–3). The wasp can also pierce through the case for oviposition and this behavior is almost equally frequent.

Molecular data. Four DNA barcodes were obtained (two of them almost full length at 631 bp, the other two specimens rendered partial barcodes of 421 bp each). Those sequences correspond to BIN BOLD:ADO8283 and are unique among all available sequences of Microgastrinae in BOLD, with the closest BIN (an undescribed *Microgaster* species from Papua New Guinea) differing by 34 bp (5.2%) (Suppl. material 4).

Etymology. The species is named after Godzilla (Japanese: ゴジラ, Hepburn: Gōjira), a fictional monster (kaiju) that became an icon after the 1954 Japanese film of the same name and many films afterwards. The wasp name is intended to respectfully honour one of the most recognizable symbols of Japanese popular culture worldwide. The wasp's parasitization behaviour bears some loose resemblance to the kaiju character, in the sense that the wasp (after diving underwater to search for its host, a moth caterpillar) suddenly emerges from the water (to parasitize the host), similar to how Godzilla suddenly emerges from the water in the movies. Additionally, Godzilla has sometimes been associated, albeit in different ways, with Mothra (Japanese: モスラ, Hepburn: Mosura) another kaiju that is typically portrayed as a larva (caterpillar) or adult moth.

The generic boundaries between *Microgaster* and *Hygroplitis*

There is some evidence that *Hygroplitis* may just be a species-group of *Microgaster* (Fernandez-Triana et al. 2020). Data based on DNA barcodes strongly suggest so (e.g., Suppl. material 4; see also Smith et al. 2013). *Hygroplitis* could represent a derived group, specialized in parasitizing aquatic or semiaquatic microlepidoptera; in fact one of the two previously known aquatic microgastrine species belongs to *Hygroplitis* (Morley, 1936). The presence in this genus of simple tarsal claws, which are elongate and strongly curved (like in Fig. 2D–F), has been suggested to be one of the main characteristics that allows hymenopterans to grip the substrate when entering the water looking for hosts (Bennett 2008).

Microgaster godzilla sp. nov. could be considered to be intermediate between those two genera but is not the first *Microgaster* reported to be like that. Another species, *Microgaster deceptor* Nixon, 1968, has similar simple tarsal claws which are relatively large, and it was also considered as intermediate between *Hygroplitis* and *Microgaster* by Nixon (1968: 55), who treated *Hygroplitis* as only a species group of *Microgaster*. Solving the generic boundaries between these genera, or perhaps synonymizing *Hygroplitis* under *Microgaster*, is beyond the scope of this paper; in any case, for the time being we prefer to place the new species within *Microgaster*.

Acknowledgements

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Supplementary material 1

Wasp parasitization behaviour including diving underwater

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Data type: multimedia

Link: https://doi.org/10.3897/jhr.79.56162.suppl1

Supplementary material 2

Wasp parasitization behaviour above water

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Data type: multimedia

Link: https://doi.org/10.3897/jhr.79.56162.suppl2

Supplementary material 3

Probing with antennae above water

Authors: Jose Fernandez-Triana, Tetsuyuki Kamino, Kaoru Maeto, Yutaka Yoshiyasu, Norio Hirai

Data type: multimedia

Link: https://doi.org/10.3897/jhr.79.56162.suppl3

Supplementary material 4

Neighbor Joining tree of all DNA barcode sequences

Authors: Jose Fernandez-Triana, Tetsuyuki Kamino, Kaoru Maeto, Yutaka Yoshiyasu, Norio Hirai

Data type: molecular data

Explanation note: Neighbor Joining tree of all DNA barcode sequences of Microgaster and Hygroplitis species in BOLD (http://www.boldsystems.org/) with more than 400 base pairs as of June 2020.

Link: https://doi.org/10.3897/jhr.79.56162.suppl4

Supplementary material 5

Differences between *Microgaster godzilla* and other *Hygroplitis* and *Microgaster* species

Authors: Jose Fernandez-Triana, Tetsuyuki Kamino, Kaoru Maeto, Yutaka Yoshiyasu, Norio Hirai

Data type: species data

Explanation note: Differences between *Microgaster godzilla* and other *Hygroplitis* and *Microgaster* species, arranged in alphabetical order.

Link: https://doi.org/10.3897/jhr.79.56162.suppl5